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Bioprospección de microorganismos haloalcolófilos aislados de un maar desecado del volcán "Hoya Rincón de Parangueo"

Bioprospecting of haloalkaliphilic microorganisms isolated from a dried-out maar in the volcano "Hoya Rincón de Parangueo"

Elizabeth Selene Gómez-Acata [1] https://orcid.org/0000-0003-0738-0483
Laura Mariana Ayala-Gámez [2]
Raúl García-Covarrubias [2]
Martha Yvette Torres-Núñez [2]
Liliana Vargas-López [2]
Susana Hernández-Camargo [2]
Itzel Guadalupe Becerril-Rojas [2]
Marcos León-Domínguez [2]
Lizbeth Zurita-García [2]
Yendi E. Navarro-Noya [3] https://orcid.org/0000-0002-7191-7815
Eloy Conde-Barajas [1] https://orcid.org/0000-0002-6098-860X
Luc Dendooven [2] https://orcid.org/0000-0002-4148-2283

[1] Tecnológico Nacional de México – Instituto Tecnológico de Celaya, ✉ mistral_1984@hotmail.com
[2] Cinvestav, Ciudad de México, Laboratorio de Ecología de Suelos
[3] CONACYT-Universidad Autónoma de Tlaxcala

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Resumen

Introducción: Los microorganismos halófilos los podemos encontrar en ambientes hipersalinos y pueden sintetizar productos de interés industrial.

Método: En el presente trabajo, 37 aislados se obtuvieron del sedimento del maar desecado de la "Hoya Rincón de Parangueo" (México) en diferentes medios de cultivo que contenían 20% de NaCl y pH de 9, se determinó el potencial de producción de enzimas extracelulares de interés industrial por estos aislados.

Resultados: Se identificaron ocho géneros bacterianos *Salsuginibacillus, Alkalibacillus, Bacillus, Staphylococcus, Oceanobacillus, Halomonas* y *Nesterenkonia* además de 15 especies diferentes de Actinobacteria, Firmicutes y Proteobacteria. Los aislados L371, LTA7 y SU3 produjeron proteasas, M20, L5, L7, L8, Y1, Y2, M3, M9 y RA lipasas, LTA4 y SU3 amilasas y LTA7, Y2, M5, M9 y RA cacesinasas a pH de 9. La producción de sideróforos se encontró en 17 aislados.

Discusión o Conclusión: Se reporta la primera evidencia experimental de la producción de sideróforos por *N. alba, Alkalibacillus* and *Oceanobacillu* a una concentración de NaCl al 6% (p/v).

Los aislados RE, RG1, RH1, RA e I11 tuvieron una distancia genética de 2.8% a 4.1% con especies de *Nesterenkonia* por lo que pueden representar nuevas especies.

Palabras clave: bioprospección; sideróforo; halófilo; lipasas; *Nesterenkonia*; industria; ambientes hipersalinos; especies; enzimas

Abstract

Introduction: Halophiles can be found in hypersaline environments and they can synthesize products of industrial interest.

Method: In this work, 37 bacterial strains were isolated from the sediment of a dried-out maar of the "Hoya Rincón de Parangueo" (Mexico) on different culture media with 20% NaCl and pH 9, while potential industrial extracellular enzymes were determined.

Results: Eight genera *Salsuginibacillus, Alkalibacillus, Bacillus, Staphylococcus, Oceanobacillus, Halomonas and Nesterenkonia* besides 15 distinct species belonging to Actinobacteria, Firmicutes and Proteobacteria were identified. The L371, LTA7 and SU3 isolates produced proteases, M20, L5, L7, L8, Y1, Y2, M3, M9 and RA lipases, LTA4 and SU3 amylases, while LTA7, Y2, M5, M9 and RA caseinases with enzymatic activity at pH 9. Production of siderophore was found in 17 isolates.

Discussion or Conclusion: It is reported for the first experimental evidence of siderophores production by *N. alba*, *Alkalibacillus* and *Oceanobacillus* at 6% NaCl (w/v). Isolates RE, RG1, RG2, RH1, RA and I11 showed 2.8% to 4.1% genetic distance with *Nesterenkonia* species and might represent new species.

Keywords: bioprospection; siderophore; halophile; lipases; *Nesterenkonia*; industry; hypersaline environments; species; enzymes

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Introduction

Hypersaline environments such as salt lakes, hypersaline ponds and saline-alkali soils (Ghai *et al*. 2011; Shi *et al*. 2012; El Hidri *et al*. 2013; Purohit *et al*. 2014) are distributed worldwide and contain a large number of microorganisms from all domains of life called halophiles, well adapted to these hostile conditions (Yin *et al*. 2015). Halophiles have been divided in two groups dependent on their tolerance to salt. Halotolerant, when they are able to grow at high salt concentrations but

do not require it for growth (Purohit *et al*. 2014) and extreme halophiles that require high salt concentrations for growth. Most halophilic bacteria belong to the Firmicutes (e.g., *Bacillus*, *Oceanobacillus*, *Alkalibacillus*, *Virgibacillus*, *Halobacillus*), Actinobacteria (e.g., *Leucobacter*, *Arthrobacter* and *Nesterenkonia*) or Proteobacteria (i.e., *Halomonas*, *Chromohalobacter* and *Halovibrio*) (Romano *et al*. 1996; Yumoto *et al*. 2005; Gonzalez-Domenech *et al*. 2009; Luo *et al*. 2009; Whon *et al*. 2010; Tang *et al*. 2011; El Hidri *et al*. 2013; Govender *et al*. 2013). Halophiles can also be alkaliphiles that grow optimally at pH 9 but are unable to grow at pH 7 or alkalitolerant with an optimal growth at a neutral pH but that can grow at pH 10 (Purohit *et al*. 2014). Haloalkaliphic microorganisms are both halophilic and alkaliphilic and live in saline alkaline environments widely distributed on earth. They can grow at pH > 10 and salt content up to 25% (w/v) (El Hidri *et al*. 2013).

There has been an increasing interest in haloalkaliphilic microorganism due to their ability to grow under extreme conditions and their enzymes that are well adapted to high pH and high salt concentration, which can be applied in industrial process. Enzymes from haloalkaliphilic bacteria can be used at high salt concentrations and alkaline pH; conditions found often in detergent, leather or silk industries in which enzymes with poor a lower stability in these conditions can not be used (Abdel-Hamed *et al*. 2016).

Halophilic and alkaliphilic microorganism can also synthesize products with industrial applications, such as polyhydroxyalkanoates (PHAs) and ectoine. The latter is an ingredient in many cosmetics, skin care products and medicinal preparations, and has been used as an enhancer in molecular biology techniques (Ma *et al*. 2010). Haloalkaliphilic bacteria produce halophilic hydrolases (proteases, xylanases, cellulases, amylases), bacteriorhodopsin, antimicrobials substances, bio-surfactants or bio-emulsifiers that have many industrial applications. For instance, proteases are applied in detergent, textile, leather, silk industries and have various pharmaceutical applications (Purohit *et al*. 2014; Yin *et al*. 2015). Siderophores are organic metal-chelating agents produced by microorganisms especially in Fe-limiting conditions. They can bind to other metals and can be used in biosensors, and as biocontrol and chelation agents (Ahmed and Holmström, 2014). However, the siderophores production by halophilic microorganisms has not been studied yet in great detail (Figueroa *et al*. 2015; Matsui and Nishino 2016).

Halophile microorganisms have not been studied as intensively as other extremophiles although they produce halophilic enzymes and secondary metabolites that can be used for

bioremediation of contaminated ecosystems, the production of polymers (Ma *et al*. 2010) and in the food industry (Purohit *et al*. 2014). It is important that enzymes used in the industry are stable when the salt and organic solvent content, pH, and temperature are high in the production process. Consequently, the isolation of enzymes from haloalkaliphilic bacteria will expand the existing industrial enzyme catalogue.

The recently dried-out maar in the volcano "*Hoya Rincón de Parangueo*" is part of the maar lakes of the Trans-Mexican Volcanic Belt. The sediment of the dried-out maar has a high pH, salt, aragonite and hydromagnesite content (Kienel *et al*. 2009), which makes it an interesting environment to search for novel extreme haloalkaliphilic microorganisms with unique metabolic capacities. The objective of this study was to isolate bacteria from this extreme haloalkaline environment that produce extracellular enzymes or siderophores with a potential industrial application.

Methods

Sampling sites and sample collection

Three sediment samples were collected from the 0-20 cm layer of a dried-out maar in the volcano "*Hoya Rincón de Parangueo*", Valle de Santiago in the state of Guanajuato, Mexico (20º 43´N, 101º 25´W) (**Fig. 1**) on July 2015, mixed and stored at 4º C until used. The study site was characterized by high electrolytic conductivity (EC) and high alkalinity (Kienel *et al*. 2009). Details of the physicochemical characteristics can be found in Ibarra-Sánchez *et al*. (2019).

Fig. 1. Inside of the dried-out maar in the volcano "Hoya Rincón de Parangueo" with whitish surface and remains of the lake.

Isolation of extremophilic bacteria on artificial growth media

Halotolerant heterothophic aerobic bacteria were grown in the liquid media A, B, C, D, E, F and G (**Supplementary Table 1**) that contained 20% NaCl. The different media were selected to enrich and isolate bacteria with a wide variety of metabolic preferences. Sediment sub-samples (10 g) were inoculated separately in Erlenmeyer flasks containing the different liquid media (90 mL) with 20% NaCl and pH 9 and incubated at room temperature and 110 rpm for 7 days except for media H, I and J (**Supplementary Table 1**). After incubation, the enriched cultures were serially diluted 10^{-4} to 10^{-6} in 15% NaCl solution and 0.1 mL aliquots were plated on Petri dishes containing each of the same agar-media. The Petri dishes were incubated at room temperature for 7 to 15 days. Sediment samples were placed on agar-medium H and J in Petri dish, while soil extract was mixed with agar in medium I. Colonies were selected based on their morphology and purified on the same media after several re-inoculations. Colonies from media F, G and I were purified in medium D due to its size were larger in this medium.

Biochemical characteristics of the isolates

Bacterial strains were examined for Gram staining, catalase and oxidase. The growth of the isolates at different concentrations of NaCl was studied by growing them in a medium containing (g L^{-1} of distilled water) peptone 5.0, yeast extract 2.0, beef extract 1.0, agar 18.0, and varying amounts of NaCl (0.0% to 35%) at pH 9, for 48 h to 72 h. The growth of the isolates at different pH was studied in liquid media containing (g L^{-1} of distilled water) $MgSO_4.7H_2O$ 1.0, NaCl 100.0, sodium citrate 3.0, yeast extract 10.0 and $(NH_4)_2SO_4$ 1.0 (Romano *et al*. 2005a) with pH ranging from 5.5 to 12 using the following buffers NaH_2PO_4/Na_2HPO_4 (pH 5.5-8.0), $NaHCO_3/Na_2CO_3$ (pH 9.0-10), $Na_2HPO_4/NaOH$ (pH 11.0-12.0) as reported by Dou *et al*. (2016). The production of different extracellular enzymes (caseinases, amylases, lipases, proteases, xylanases and cellulases) and siderophore production were tested. The different media contained ingredients as listed in **Supplementary Table 2**. Production of extracellular enzymes were determined after 24 h to 72 h and siderophore production were confirmed by a yellow halo around the colonies.

DNA extraction and PCR amplification

The DNA was extracted from pure cultures after 48 h at room temperature. The cells were harvested with 15% w/v NaCl and centrifuged at 7,700 × *g* for 15 min. The pellets were dissolved in one mL

buffer (0.25 M EDTA pH 8), 80 µL 10 mg mL^{-1} lysozyme was added and the mixture was incubated at 37 ºC for one h. One mL lysis solution (0.1M NaCl, 0.5 M Tris-HCl [pH 8], 12% sodium dodecyl sulphate) and 0.5 g sterile sand were added, and samples were vortexed for 10 min. The solution was treated with a cycle of freezing at -70 ºC for 20 min and thawing at 65 ºC for 20 min or 30 s at 6.0 m s^{-1} in FastPrep-24™ (Biomedicals, Solon, OH). The sample was centrifuged at 7,700 × *g* at room temperature for 10 min. Proteins were removed from the supernatant with 1/5 volume EDTA (0.5 M, pH 8) and 1/10 volume potassium acetate (5 M, pH 5.5), incubated at 4º C for 20 min and centrifuged at 12,000 × *g* at 4 ºC for 5 min. The supernatant was extracted twice with 500 µL chloroform-isoamyl alcohol (24:1 vol: vol). The solution was transferred to a clean tube and DNA precipitated with one volume 13% polyethylene glycol (MW, 8000) dissolved in 1.6 M NaCl. The mixture was incubated at -20 ºC overnight and centrifuged at 12,000 × *g* at 4 ºC for 15 min. The DNA pellet was washed with cool 70% ethanol, dried and dissolved with 50 µL sterile deionized water and stored at -20 ºC.

The 16S rRNA genes of the genomic DNA were amplified with primers 27F and 1492R (Lane, 1991). The reaction mixture contained 2 mM $MgCl_2$, 0.2 mM each dNTP, 10 µM of each primer, 10 ng DNA template and 0.2 U *Taq* DNA polymerase (Thermo Scientific, California, USA) with 1 × reaction buffer in a total volume of 25 µL. The PCR was done with an initial denaturation at 95ºC for 10 min, 30 cycles of 45 s at 95 ºC, 45 s at 56 ºC and 60 s at 72 ºC, followed by a final 10 min extension at 72 ºC. The PCR products were purified using UltraClean® PCR Clean-Up kit (MoBio Laboratories, Carlsbad, CA, USA) as recommended by the manufacturer. Sequencing was done by Macrogen Inc. (DNA Sequencing Service, Seoul, Korea) in an ABI 3730XLs sequencer.

Sequence analysis

All the sequences were compared with 16S rRNA gene sequences available in the EZBioCloud database (http://www.ezbiocloud.net/, 17 july 2018). Pairwise and multiple sequence alignments of approximately 1300 bp were done using CLUSTAL W 2.0 (Larkin *et al*. 2007). A phylogenetic tree was constructed from evolutionary distances using the neighbour-joining clustering method. The best substitution model to calculate the genetic distances was determined with the Model Test implemented in MEGA X (Kumar *et al*. 2018). Tree topologies were determined by bootstrap analysis of 1000 replications in MEGA X package. The 16S rRNA gene sequences obtained have

been submitted to the NCBI GenBank Database under accession numbers as MH727817 to MH727853.

Results

16S rRNA phylogenetic analysis

Thirty-seven strains were obtained from the dried-out maar in the volcano "Hoya Rincón de Parangueo". Twenty-four % of the isolates grew in low nutrient medium whereas 76% were isolated in high rich medium (**Supplementary Table 3**). The majority of the isolates belonged to the Firmicutes (23 isolates) followed by Proteobacteria (eight isolates) and Actinobacteria (six isolates) (**Fig. 2**). Media A, B, C, D, E, F, I and J favoured the isolation of Firmicutes, media G and H Proteobacteria, while Actinobacteria were obtained only in the medium I (**Supplementary Table 3**). The isolates were classified in eight bacterial genera. The most abundant genera were *Alkalibacillus* (*A. haloalkaliphilus, A. filiformis*) (**Fig. 3a**) and *Halomonas* (*H. pantelleriensis* and *H. fontilapidosi)* (eight isolates each one) (**Fig. 3b**) followed by *Nesterenkonia* (*N. lutea and N. alba*) (six isolates) (**Fig. 3c**), *Oceanobacillus* (*O. oncorhynchi and O. kimchii*) (five isolates), *Staphylococcus* (*S. equorum* subsp. *equorum*) (four isolates), *Bacillus* (*B. luteus, B. lindianensis and B. chagannorensis, B. saliphilus)* (four isolates) and *Salsuginibacillus* (*S. halophilus)* (two isolates) (**Fig. 3a**).

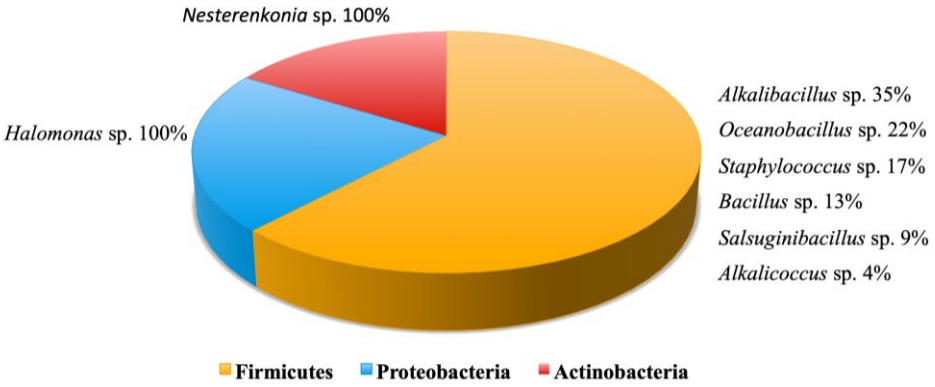

Fig. 2. Bacteria isolated from the sediment of a dried-out maar in the volcano "Hoya Rincón Parangueo", Guanajuato, Mexico at the phylum and genus level.

Isolates RE (95.9%), RG1 (95.9%), RG2 (96.4%), RH1 (96.9%), RA (96.0%) and I11 (95.7%) belonged to *Nesterenkonia* and might be new species, as they had more than 3% of interspecific divergence and less than 2% intraspecific divergence (**Fig. 3c**, **Supplementary Table 4**).

Fig. 3a. Phylogenetic diversity of haloalkaliphilic heterotrophic Bacteria based on comparison of 16S rRNA gene of isolates from the sediment of a dried-out maar in the volcano “*Hoya Rincón Parangueo*”, Guanajuato, Mexico with representatives of the EzBiocloud database. a) Members of phylum Firmicutes. The tree was constructed with Maximum Likelihood method. The evolutionary distances were computing using 1298 bp, 1396 bp and 1300 bp using Tamura 3-parameter model for Firmicutes, Proteobacteria and Actinobacteria, respectively. The rate variation among sites was modelled with gamma distribution (shape parameter=5). All positions containing gaps and missing data were eliminated. The numbers of the branches represent percentages of bootstrap sampling of 1000 replications of neighbour-joining/maximum likelihood analysis. * Produce siderophore, ◆ produce lipase, O produce amylase, ❖ produce caseinase, growth above pH 8, ⊙ growth above 5% NaCl. Strains in green bold are possible new species.

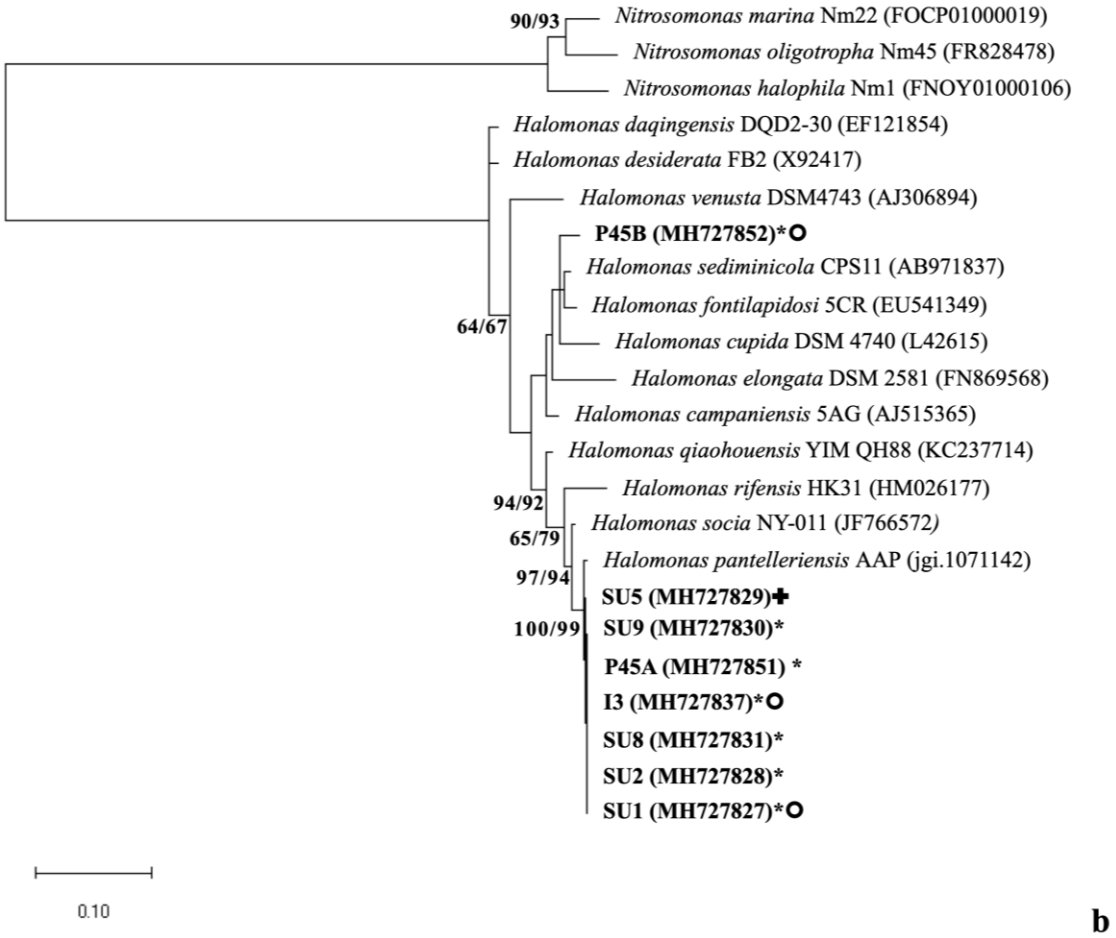

Fig. 3b. Phylogenetic diversity of haloalkaliphilic heterotrophic Bacteria based on comparison of 16S rRNA gene of isolates from the sediment of a dried-out maar in the volcano "*Hoya Rincón Parangueo*", Guanajuato, Mexico with representatives of the EzBiocloud database. b) Members of phylum Proteobacteria.

The tree was constructed with Maximum Likelihood method. The evolutionary distances were computing using 1298 bp, 1396 bp and 1300 bp using Tamura 3-parameter model for Firmicutes, Proteobacteria and Actinobacteria, respectively. The rate variation among sites was modelled with gamma distribution (shape parameter=5). All positions containing gaps and missing data were eliminated. The numbers of the branches represent percentages of bootstrap sampling of 1000 replications of neighbour-joining/maximum likelihood analysis. * Produce siderophore, ◆ produce lipase, O produce amylase, ❖ produce caseinase, growth above pH 8, ⊙ growth above 5% NaCl. Strains in green bold are possible new species.

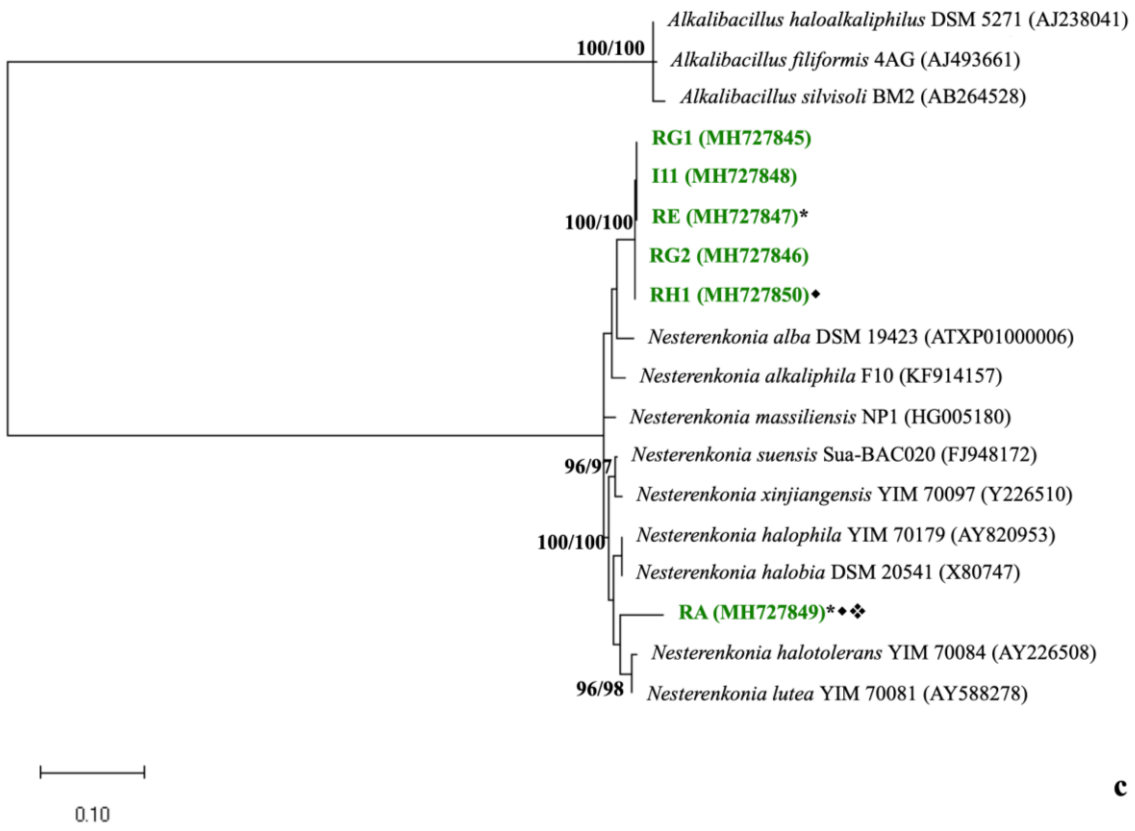

Fig. 3c. Phylogenetic diversity of haloalkaliphilic heterotrophic Bacteria based on comparison of 16S rRNA gene of isolates from the sediment of a dried-out maar in the volcano "*Hoya Rincón Parangueo*", Guanajuato, Mexico with representatives of the EzBiocloud database. c) Members of phylum Actinobacteria.

The tree was constructed with Maximum Likelihood method. The evolutionary distances were computing using 1298 bp, 1396 bp and 1300 bp using Tamura 3-parameter model for Firmicutes, Proteobacteria and Actinobacteria, respectively. The rate variation among sites was modelled with gamma distribution (shape parameter=5). All positions containing gaps and missing data were eliminated. The numbers of the branches represent percentages of bootstrap sampling of 1000 replications of neighbour-joining/maximum likelihood analysis. * Produce siderophore, ◆ produce lipase, ❍ produce amylase, ❖ produce caseinase, growth above pH 8, ⊙ growth above 5% NaCl. Strains in green bold are possible new species.

Haloalkaliphilic bacteria isolation and biochemical characteristics

All isolates were catalase positive, oxidase negative except for isolates LTA7, RH2, Y1, P45A, I3, P45B and SU8, 76% were Gram-positive and 24% Gram-negative (**Supplementary Table 3**). All isolates grew optimally at pH 9, 24% of them could growth only at pH > 8 and 49% could growth at pH < 8 (**Supplementary Table 5**, **Fig. 4a**). All isolates grew in more than 10% NaCl, most of them grew with less than 5% NaCl and only two grew in 30% NaCl, but more than 80% could growth in 25% NaCl and 59% grew with no salt addition to the medium (**Supplementary Table 5**, **Fig. 4b**).

Haloalkaliphilic Bacteria Enzymatic activities

The bacterial isolates (37) were screened for extracellular amylases, cellulases, xylanases, caseinases, chitinases, proteases and lipases (**Fig. 4c**). Overall, 20 isolates (53%) produced at least one of these enzymes, but none showed activity for all of them. Amylase activity was detected in 19% of isolates belonging to *A. haloalkaliphilus*, *B. chagannorensis*, *B. lindianensis*, *B. luteus*, *H. pantelleriensis* (two isolates) and *H. fontilapidosi*. Lipase activity was found in 10 isolates belonging to *S. equorum* (four isolates), *O. oncorhynchi*, *O. kimchii* (three isolates) *N. alba* and *N. lutea*. Moreover, caseinase activity was detected in 16% of the isolates i.e., *A. saliphilus*, *O. kimchii* (three isolates) *N. lutea* and *B. lindianensis* (**Supplementary Table 5**).

Siderophore production

Siderophore production was found in 17 isolates (46%), belonging to Firmicutes (22%), Proteobacteria (19%) and Actinobacteria (5%) (**Fig. 4c**). They were members of *S. equorum* (four isolates), *A. haloalkaliphilus*, *A. filiformis*, *O. kimchii* (two isolates), *H. pantelleriensis* (six isolates), *H. fontilapidosi*, *N. alba* and *N. lutea* (**Supplementary Table 5**).

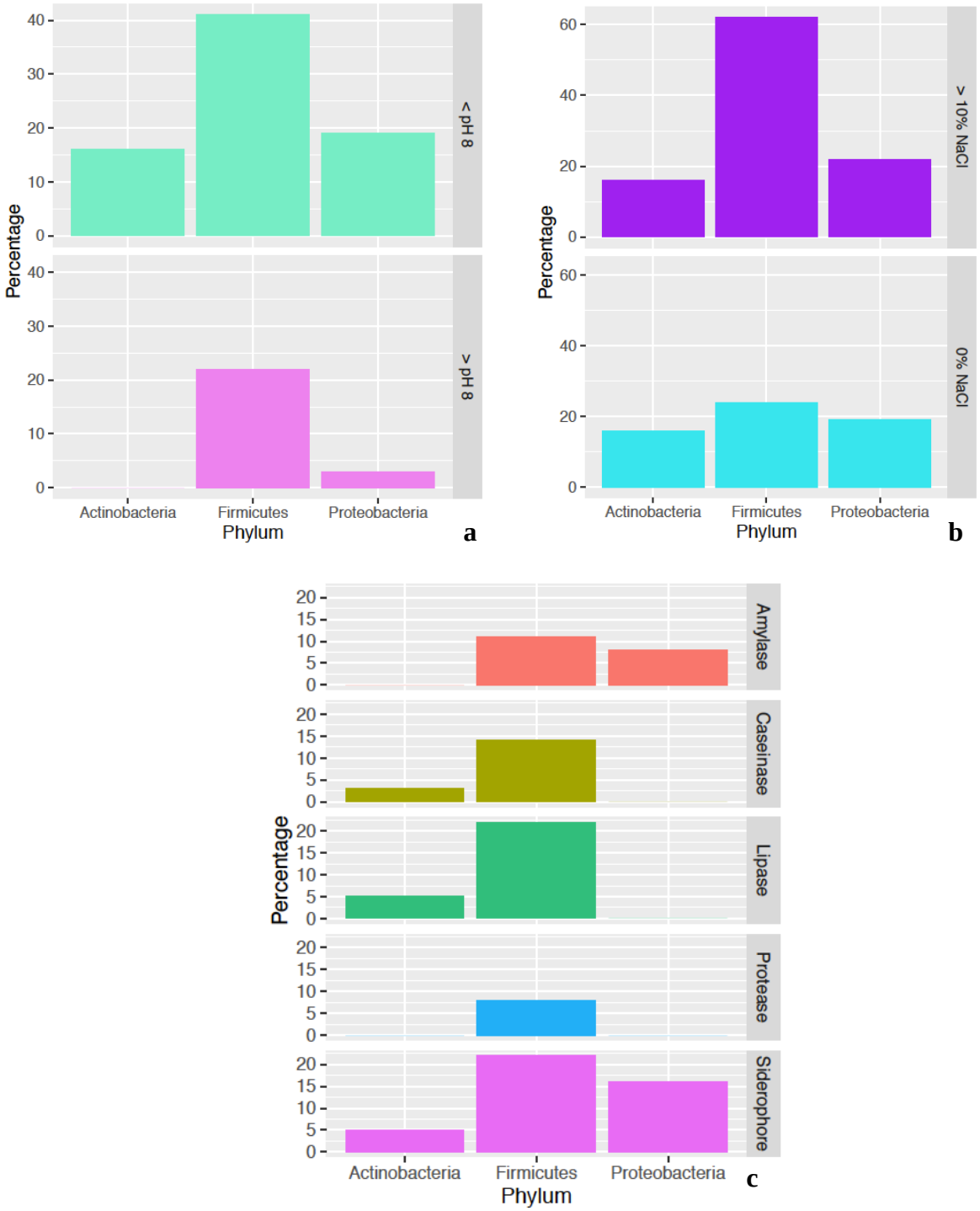

Fig. 4. Percentage of isolates that grow at different a) pH, b) salt concentration and c) enzyme detection of bacterial phyla isolated from the sediment of a dried-out maar in the volcano “Hoya Rincón Parangueo”, Guanajuato.

Discussion

Halotolerant microorganisms have been isolated from hypersaline environments, such as hypersaline ponds (Tang *et al*. 2011), saline soil (Shi *et al*. 2012) and different other saline environments (El Hidri *et al*. 2013). However, no reports exist about the isolation of haloalkaliphilic microorganisms from a dried-out maar of a volcano, such as the "Hoya Rincón de Parangueo". In this study, isolates from Firmicutes were more abundant than those belonging to the Proteobacteria and Actinobacteria as found in other hypersaline environments, such as a saline-alkali soil from China (Tang *et al*. 2011; Shi *et al*. 2012) and a saline lake from India (Joshi *et al*. 2008).

The ability to produce enzymes with industrial application was tested. Lipases, which are widely used in detergent and food industry (Panda and Gowrishankar, 2005), were found only in *O. oncorhynchi*, *O. kimchii* and *N. lutea*. The production of lipases has been reported in halophiles, such as *Staphylococcus, Oceanobacillus and Nesterenkonia* (El Hidri *et al*. 2013; Jeong *et al*. 2014; Kiran *et al*. 2014). However, there are no reports of lipase production by species found in this study. Amylases, which are in increasing demand for their application in detergent, pharmaceutical production and wastewater treatment (Margesin and Schinner, 2001), were detected in isolates belonging to *Alkalibacillus*, *Bacillus* and *Halomonas* as reported previously (Romano *et al*. 1996; Jeon *et al*. 2005; Gonzalez-Domenech *et al*. 2009; El Hidri *et al*. 2013). Although caseinase activity has been reported in *Bacillus*, *Oceanobacillus* and *Nesterenkonia* (Romano *et al*. 2005b; Delgado *et al*. 2006; Hirota *et al*. 2013), there are no reports of caseinase production by *O. kimchii* and *N. lutea* as found in this study. They might be alternatives for halophilic proteases applied as laundry additives, and in pharmaceutical waste management and food processing (Razzaq *et al*. 2019).

Siderophores have many applications in agriculture, bioremediation techniques, microbial ecology and medicine, i.e., they are used as biocontrol agents, biosensors, to improve the mechanism of antibiotics and to improve the growth of uncultured microorganisms in the laboratory (Ahmed and Holmström, 2014; Saha *et al*. 2016). Siderophore production by microorganisms is well documented (Cabaj and Kosakowska, 2009; Peek *et al*. 2012; Lehner *et al*. 2013) and studies have focused mainly on *Staphylococcus* sp. due to its clinical importance (Dale *et al*. 2004; Mukherjee *et al*. 2017). Halophiles and alkaliphiles, such as *Bacillus* sp. and *Halomonas* sp., produce siderophores (Figueroa *et al*. 2015; De Serrano *et al*. 2016; Matsui and Nishino, 2016) and siderophores of *N. jeotgali*, *N. massiliensis* and *Nesterenkonia* sp. are involved

in mechanisms of pathogenicity through iron acquisition (Chander *et al*. 2017). However, no evidence of siderophore production by *N. alba, Oceanobacillus* and *Alkalibacillus* have been reported previously.

Bacillus sp., *Nesterenkonia* sp., *Halobacillus* sp., *Filobacillus* sp., *Micrococcus* sp., *Salinivibrio* sp., *Halomonas* sp., *Streptomyces* sp. and *Virgibacillus* sp. are halophiles known to produce halophilic hydrolases with industrial applications. In this study, *Alkalibacillus* and *Halomonas* were the most abundant genera with a known biotechnological application as they produce ectoine, PHA and hydrolases (Bergmann *et al*. 2013; Yin *et al*. 2015). Additionally, some of the microorganisms isolated in this study produce siderophores and amylases with promising industrial application.

Members of *Nesterenkonia* was the third most abundant genus isolated from the dried-out maar in the volcano "Hoya Rincón de Parangueo". Some strains belonging to *Nesterenkonia* are haloalkaliphilic found in hypersaline natural environments, industry effluents and fermented seafood (Chander *et al*. 2017). At the time of writing, the genus *Nesterenkonia* include 20 species with validated names with 14 of them isolated from natural hypersaline environments, i.e. *N. halobia* (Mota *et al*. 1997), *N. lacusekhoensis* (Collins *et al*. 2002), *N. xinjiangensis, N. halotolerans* (Li *et al*. 2004), *N. lutea, N. sandarakina* (Li *et al*. 2005), *N. aethiopica* (Delgado *et al*. 2006), *N. halophila* (Li *et al*. 2008), *N. rhizosphaerae* (Wang *et al*. 2014), *N. alkaliphila* (Zhang *et al*. 2015), *N. aurantica* (Finore *et al*. 2016), *N. cremea* (Sultanpuram *et al*. 2017), *N. pannonica* (Borsodi *et al*. 2017) and *N. natronophila* (Machin *et al*. 2019). Isolates found in this study are grouped with *N. alba*, a strain isolated from black liquor of a cotton pulp (Luo *et al*. 2009). Members of *Nesterenkonia* are known to produce halophilic proteases and xylanases (Yin *et al*. 2015), but no evidence of siderophore production has been reported as found in this study. Based on a cut-off of 97% identity for 16S rRNA gene, commonly used to discriminate bacterial species (Tindall *et al*. 2010), the isolates from the cultured *Nesterenkonia* could be new species. Further studies will be necessary to get the physiological and biochemical characteristics of these isolates to differ them from the recognized species.

Finally, isolates obtained from "Hoya Rincón de Parangueo" can grow at very different pH, temperature, and salt concentration compare with those found in other volcano craters in Mexico, such as "El Chichón" where acidophilic bacteria were isolated (Ovando-Chacón, *et al*. 2020) or Xinantécatl volcano where psychrophilic microorganisms are found (Tapia-Vázquez *et al*. 2020).

However, lipase production was detected in bacteria from both “Hoya Rincón de Parangueo” and "El Chichón" despite the pH difference of each environment. Moreover, siderophore production was found in isolates from the Xinantécatl volcano, a cold environment, as well as in this study.

In conclusion, strains isolated from the dried-out maar in the volcano “Hoya Rincón de Parangueo” were haloalkaliphilic or halotolerant. Six isolates belonging to genus *Nesterenkonia* could be new species. Siderophore production was detected for the first time in extreme microorganisms belonging to *N. alba*, *Alkalibacillus* and *Oceanobacillus.*

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Conflict of interest

The authors declare that they have no conflict of interest.

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